ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 2007, p. 4022–4028 Vol. 51, No. 11
0066-4804/07/$08.00+0 doi:10.1128/AAC.01259-06

Molecular Epidemiology of Clinical Isolates of Carbapenem-Resistant *Acinetobacter* spp. from Chinese Hospitals▽

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Received 8 October 2006/Returned for modification 10 December 2006/Accepted 29 August 2007

Carbapenem resistance in *Acinetobacter* spp. is an emerging problem in China. We investigated the molecular epidemiology and carbapenemase genes of 221 nonrepetitive imipenem-resistant clinical isolates of *Acinetobacter* spp. collected from 1999 to 2005 at 11 teaching hospitals in China. Genotyping by pulsed-field gel electrophoresis (PFGE) found 15 PFGE patterns. Of these, one (clone P) was identified at four hospitals in Beijing and another (clone A) at four geographically disparate cities. Most imipenem-resistant isolates exhibited high-level resistance to all β-lactams and were only susceptible to colistin. bla_{OXA-23}-like genes were found in 97.7% of isolates. Sequencing performed on 60 representative isolates confirmed the presence of the bla_{OXA-23} carbapenemase gene. Analysis of the genetic context of bla_{OXA-23} showed the presence of IS*Aba1* upstream of bla_{OXA-23}. All of the 187 *A. baumannii* isolates identified by amplified RNA gene restriction analysis carried a bla_{OXA-51}-like oxacillinase gene, while this gene was absent from isolates of other species. Sequencing indicated the presence of bla_{OXA-66} for 18 representative isolates. Seven isolates of one clone (clone T) carried the plasmid-mediated bla_{OXA-58} carbapenemase gene, while one isolate of another clone (clone L) carried the bla_{OXA-72} carbapenemase gene. Only 1 isolate of clone Q carried the bla_{IMP-8} metallo-β-lactamase gene, located in a class 1 integron. Of 221 isolates, 77.8% carried bla_{PER-1}-like genes. Eleven different structures of class 1 integrons were detected, and most integrons carried genes mediating resistance to aminoglycosides, rifampin, and chloramphenicol. These findings indicated clonal spread of imipenem-resistant *Acinetobacter* spp. and wide dissemination of the OXA-23 carbapenemase in China.

Acinetobacter spp. are important opportunistic pathogens responsible for a variety of nosocomial infections, including ventilator-associated pneumonia, bacteremia, surgical-site infections, secondary meningitis, and urinary tract infections. Treatment of *Acinetobacter* infections is often complicated by multidrug-resistant phenotypes, including resistance to broad-spectrum β-lactams, aminoglycosides, and fluoroquinolones. Carbapenems are currently the antibiotics of choice against multidrug-resistant *Acinetobacter* infections. However, carbapenem resistance is increasingly being reported and has become a significant public health concern, leaving few therapeutic options (7, 9, 13, 14, 16, 23, 30). Acquired carbapenem resistance in *Acinetobacter* is often associated with acquired carbapenemase production, including production of the IMP-, VIM- and SIM-type metallo-β-lactamases or the OXA-23-, OXA-24-, and OXA-58-type class D carbapenemases (3, 18). Overproduction of the natural oxacillinase (OXA-51 type) can also be associated with acquired carbapenem resistance in *Acinetobacter baumannii* (18).

In China, national resistance surveillance data from intensive care units (ICU) at 19 teaching hospitals (1996 to 2002) showed that 5% of *Acinetobacter* isolates were resistant to imipenem (25). However, another national surveillance program involving 10 geographically disparate hospitals found that resistance to carbapenems increased from 4.5% in 2003 to 18.2% in 2004 (26). At Peking Union Medical College Hospital (PUMCH), a 1,600-bed tertiary care teaching hospital, from 1993 to 2003, 5% of *A. baumannii* isolates were resistant to imipenem. However, in 2004, the number of imipenem-resistant isolates increased rapidly, with more than 50% of ICU isolates exhibiting the resistant phenotype, compared to 20% at non-ICU wards. These isolates were coresistant to other commonly used antimicrobial agents, including ampicillin-sulbactam, ceftazidime, cefepime, piperacillin-tazobactam, ciprofloxacin, and amikacin (H. Wang, unpublished data).

The objectives of the present study were (i) to investigate the molecular epidemiology of carbapenem-resistant *Acinetobacter* at several large teaching hospitals in China and (ii) to characterize the carbapenem resistance mechanisms of the major epidemic clones.

MATERIALS AND METHODS

Bacterial isolates. A total of 221 nonrepetitive imipenem-resistant clinical isolates of *Acinetobacter* spp. were collected from 11 teaching hospitals, including 117 isolates from PUMCH from 1999 to 2005 and 104 isolates from the 10 remaining centers from 2002 to 2005. The regional and time distributions of the imipenem-resistant *Acinetobacter* sp. isolates are summarized in Table 1. The isolates were identified by conventional techniques and automated systems, including a Vitek system (Vitek compact; bioMérieux Vitek Systems Inc., Hazelwood, MO) and a Phoenix 100 system (Becton Dickinson and Company, Sparks, MD) at PUMCH.

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▽ Published ahead of print on 10 September 2007.

TABLE 1. The regional and time distributions of imipenem-resistant *Acinetobacter* spp. collected from 11 teaching hospitals from 1999 to 2005 in this study

Hospital[a]	City	Location in China	No. of isolates collected in:						
			1999	2000	2001	2002	2003	2004	2005
PUMCH	Beijing	North	6	1	1	1	2	76	30
CYH	Beijing	North					7		2
FWH	Beijing	North					3		
BJ3	Beijing	North					2	11	19
BJH	Beijing	North							10
GZH	Guangzhou	South				8	10		
FZH	Fuzhou	Southeast					1	1	
JNH	Jinan	North						2	
DLH	Dalian	Northeast							2
ZJH	Hangzhou	Southeast						9	15
NJH	Nanjing	Southeast							2

[a] CYH; Beijing Chaoyang Hospital; GZH, Guangzhou Zhongshan No. 1 Hospital; FZH, Fuzhou Xiehe Hospital; JNH, Shandong Qilu Hospital; DLH, Dalian No. 1 Medical College Hospital; NJH, Nanjing Provincial People Hospital.

Molecular taxonomic identification. DNAs were extracted by incubating pure bacterial suspensions at 95°C for 10 min, and removal of debris was done by centrifugation for 10 min at 12,000 × *g*. Genomic identification was carried out by amplified rRNA gene restriction analysis (ARDRA) as previously described (10).

Antimicrobial susceptibility testing. MICs were determined by the agar dilution method of the Clinical and Laboratory Standards Institute (CLSI) (5) and interpreted according to the CLSI standards (6). Antimicrobials were supplied and stored according to the manufacturer's instructions. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as reference strains for susceptibility testing.

IEF. Crude cell extracts were prepared by three sonications in 0.1 M phosphate buffer (pH 7.0) for 30 s each time with intermittent 15-s cooling periods on ice with a Branson sonicator (Sonifier cell disruptor model W185), followed by removal of cellular debris by centrifugation at 12,000 × *g* at 4°C for 30 min. Isoelectric focusing (IEF) analysis was performed on polyacrylamide gels (pH 3.5 to 9.5; Amersham Pharmacia Biotech, Piscataway, NJ) according to the manufacturer's instructions. β-Lactamase activity was visualized by staining the gels with nitrocefin (150 μM) (Becton Dickinson). Strains producing TEM-1 (pI 5.4), TEM-10 (pI 5.6), SHV-12 (pI 8.2), and CMY-2 (pI 9.0) were used as IEF controls.

Conjugation experiment. Transfer of imipenem resistance was studied by performing conjugation experiments as previously described (27). *E. coli* C600 (Lac^- Nal^r Rif^r) was used as the recipient for the conjugation experiment. Transconjugants were selected on Trypticase soy agar containing 4 μg of imipenem, 50 μg of nalidixic acid, and 60 μg of rifampin per ml.

Molecular typing by PFGE. For pulsed-field gel electrophoresis (PFGE), ApaI-digested genomic DNA was prepared according to the manufacturer's instructions (Bio-Rad Laboratories, Hercules, CA), and restricted fragments were separated on a CHEF MAPPER system (Bio-Rad) for 20 h at 14°C with 5 to 8 s of linear ramping at 6 V/cm. DNA fingerprints were interpreted as recommended by Tenover et al. (19).

PCR and sequencing of β-lactamase genes. A multiplex PCR assay was used to detect four groups of OXA carbapenemase genes, including bla_{OXA-23}-like, bla_{OXA-24}-like, bla_{OXA-58}-like and bla_{OXA-51}-like genes, as recently described (28). The entire bla_{OXA-23}-like, bla_{OXA-24}-like, bla_{OXA-51}-like, and bla_{OXA-58}-like coding regions were amplified and sequenced using primer pairs as previously described (1, 11, 12). Genes coding for Ambler class B carbapenemases and class A serine enzymes were detected by PCR using primers specific for bla_{IMP}, bla_{VIM} (20), bla_{TEM}, bla_{SHV}, bla_{CTX-M} (27), bla_{GES}, bla_{VEB}, and bla_{PER} (4). IS*Aba1* was sought, and PCR mapping experiments using combinations of the IS*Aba1* primers and reverse primers designed against the OXA-23-like, OXA-51-like and OXA-58-like genes were carried out as described by Turton et al. (21). PCR amplification of class 1 integrons and analysis of the genetic context of resistance genes were performed on genomic DNA as described previously (15).

PCR products were purified by using a QIAquick PCR purification kit (QIAGEN, Hilden, Germany). DNA sequencing was performed by the direct sequencing method with an ABI Prism 3100 genetic analyzer (Applied Biosystems, Foster City, CA). Internal primers were designed to sequence the entire coding regions of the bla_{OXA-23}-like, bla_{OXA-24}-like, bla_{OXA-51}-like, and bla_{OXA-58}-like genes. Similarity searches and alignments for both the nucleotide sequences and predicted protein sequences were performed with the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST).

Plasmid detection and Southern hybridization. Plasmid DNA was extracted with a QIAGEN plasmid Miniprep kit (QIAGEN, Hilden, Germany) according to the manufacturer's recommendations. *E. coli* V517 (plasmid sizes, 54, 5.6, 5.1, 3.9, 3.0, 2.7, and 2.1 kb) and *E. coli* J53 containing plasmid R1 (92 kb) or R27 (182 kb) were used as standards. The sizes of plasmids were calculated by using Quantity One software (Bio-Rad). The bla_{OXA-23}-like, bla_{OXA-58}-like, and bla_{OXA-51}-like PCR products were labeled by supplementing the master mixture with Dig-dUTP (Roche Applied Science, Mannheim, Germany). Southern hybridization and detection steps were accomplished using a Dig-dUTP detection kit as recommended by the manufacturer (Roche Applied Science, Mannheim, Germany).

Collection of clinical data. The medical records of all patients identified as having imipenem-resistant *Acinetobacter* isolates at PUMCH in 2004 were reviewed by a physician. The following variables were collected: age, gender, length of stay in the ICU, ward transfer, underlying diseases, APACHE II scores, use of mechanical ventilation, and antibiotic use 15 days or less prior to the isolation.

Nucleotide sequence accession numbers. The nucleotide sequences of the bla_{OXA-72}, bla_{OXA-66}, bla_{OXA-58}, bla_{OXA-23}, and bla_{PER-1} genes have been deposited in the GenBank sequence database and were assigned the accession numbers EF534256, EF534257, EF534258, EF534259, and EF535600, respectively.

RESULTS

Molecular taxonomic identification. All 221 imipenem-resistant isolates were analyzed by ARDRA. Of these, 187 isolates were identified as *A. baumannii*. The remaining 34 isolates were identified as belonging to genospecies 10/11 (n = 18), genospecies 3 (n = 2), genospecies 13TU (n = 5), *Acinetobacter phenon* 5 (n = 3), and *A. phenon* 6/ct 13TU (n = 6).

Molecular epidemiology of imipenem-resistant *Acinetobacter* isolates. All 221 imipenem-resistant isolates were genotyped by ApaI digestion-PFGE analysis and categorized into 15 defined pulsotypes, with two or more subtypes for some of them. Table 2 shows the distribution of the 15 PFGE clones at 11 teaching hospitals from 1999 to 2005. During the study period, clonal spread occurred at 10 hospitals. These hospitals had one to three disseminated imipenem-resistant clones, most of which circulated and persisted for several years within the hospital wards.

All isolates of clones P and A were identified as *A. baumannii* by ARDRA. These two clones were the most prevalent and were found at several hospitals. However, while clone P was

TABLE 2. PFGE clone distribution of 221 imipenem-resistant *Acinetobacter* spp. at 11 teaching hospitals from 1999 to 2005

Year	PFGE type and subtype (no. of isolates) from:[a]										
	PUMCH	CYH	FWH	BJ3	BJH	GZH	FZH	JNH	DLH	ZJH	NJH
1999	A1 (4) B (2)										
2000	B (1)										
2001	A1 (1)										
2002	C (1)					M (4) N (4)					
2003	A1 (2)	E (4) F (3)	P1 (3)	P1 (2)		M (2) N (8)	Q (1)				
2004	P1 (50) P2 (18) P3 (6) A1 (1) R (1)			P1 (7) P3 (4)			V (1)	U (2)		A3 (5) T1 (4)	
2005	P1 (20) P2 (2) G (5) A1 (2) L (1)	F (2)		P1 (17) P3 (2)	P2 (10)				A2 (2)	A3 (4) T1 (8) T2 (3)	A4 (2)

[a] CYH; Beijing Chaoyang Hospital; GZH, Guangzhou Zhongshan No. 1 Hospital; FZH, Fuzhou Xiehe Hospital; JNH, Shandong Qilu Hospital; DLH, Dalian No. 1 Medical College Hospital; NJH, Nanjing Provincial People Hospital.

only detected in Beijing City, clone A was found in four cities, including Beijing, Hangzhou, Dalian, and Nanjing. In 2003, five clone P isolates were found in two hospitals (Beijing Fuwai Hospital [FWH] and Beijing 304 Hospital [BJ3]), and the number of clone P isolates increased to 136 in 2004, involving three hospitals (PUMCH, BJ3, and Beijing Hospital [BJH]) in Beijing City (Table 2). The biggest outbreak caused by clone P occurred at PUMCH. The first imipenem-resistant clone P isolate was isolated from the pleural fluid of a patient admitted to the ICU of PUMCH from a small hospital near Beijing in February 2004. Since then, clone P isolates have been found in 19 different wards throughout the hospital. Despite the fact that infection control measures have been established since May 2004, this clone still existed in nine wards in 2005.

The review of the clinical data for the 74 cases yielding clone P isolates revealed that 33 cases were pulmonary infections and 15 cases were bloodstream infections, with crude mortality rates of 22.1% and 40.2%, respectively. The remaining 28 cases were classified as colonized on the basis of the evaluation of the clinical chart. For all 74 cases, the average length of stay in the hospital before the isolation of clone P was 27.8 days. All patients had severe underlying disease, and >70% had received broad-spectrum antimicrobials and been subjected to mechanical ventilation.

Clone A, which was the predominant clone prior to 2003, persisted at PUMCH for 6 years. This clone was also found at three other hospitals in three different Chinese cities (Table 2). Transfer of patients among these four hospitals could not be documented.

Compared to other hospitals, Zhejiang University No. 1 Medical College Hospital [ZJH] had more isolates representing clone A (Table 2). Two out of 4 clone A isolates and 8 out of 11 clone T isolates were collected from blood samples taken at its surgical ICU, medical ICU, or cardiac surgery wards from March to October 2005.

Antimicrobial susceptibility. The MICs of 13 antimicrobial agents were determined for all 221 isolates. All 221 isolates were resistant to imipenem and meropenem (the MICs of both agents ranged from 16 to 128 μg/ml; the MIC for 90% of the strains tested was 64 μg/ml). Most of the isolates, but not clone L (*Acinetobacter* genospecies 3), exhibited high resistance to piperacillin-tazobactam and cefepime. In some cases, isolates of the same PFGE clone had different resistance patterns even at the same hospital. The 26 representative isolates shown in Table 3 were selected according to PFGE type, hospital source, and antimicrobial susceptibility pattern for further characterization (see below). Most non-*A. baumannii* clones, such as B, G, and C, were resistant to rifampin but susceptible to amikacin, fluoroquinolones, and minocycline (Table 3). The MICs of levofloxacin for all isolates were lower than those of ciprofloxacin. No isolate was resistant to colistin.

IEF. The 26 representative isolates selected for further investigation were subjected to IEF analysis for the detection of β-lactamases. Twenty-five of them, but not clone L (isolate PU78), produced β-lactamases that focused with a pI of >9.0, presumably representing the endogenous cephalosporinase. Most representative isolates produced multiple enzymes, including β-lactamases possessing TEM-like or PER-like pI values (pI range, 5.4 to 6.0) and others possessing pI values of 6.6 to 8.2 (Table 4).

Conjugation experiment. Imipenem resistance was not conjugatively transferred to *E. coli* C600 for any of the 26 representative isolates.

β-Lactamase genes. PCRs for the detection of different β-lactamase genes were performed with all 221 isolates. The $bla_{\text{OXA-23}}$-like gene was the most prevalent, being detected in 97.7% (216/221) of the isolates. The entire $bla_{\text{OXA-23}}$-like gene sequence was determined in 60 isolates (including the representative isolates in Table 4), which confirmed the presence of the $bla_{\text{OXA-23}}$ gene. In contrast to the other clone A isolates, PFGE A4 subtype isolates such as NJ59 did not carry the OXA-23 gene.

$bla_{\text{OXA-51}}$-like genes were found in 187 isolates (belonging to 7 clones), all of which were identified as *A. baumannii*.

TABLE 3. MICs of 13 antimicrobial agents for 26 representative isolates of 15 clones at different hospitals

Hospital[a]	PFGE clone(s)	No. of isolates	Representative isolate	MIC (μg/ml) of:[b]												
				IPM	MEM	PTZ	FEP	CAZ	CSL[c]	GEN	AMK	CIP	LVX	RIF	MIN	POL
PUMCH	P1	61	PU4A1	64	64	>256	>256	>256	64	>256	>256	16	4	4	2	0.5
	P1/P2/P3	35	PU73	64	64	>256	>256	>256	32	>256	>256	>32	16	4	8	1
	A1	10	PUA1	64	64	>256	>256	>256	64	>256	8	>32	16	32	4	0.5
	B	3	PUA6	64	64	>256	>256	>256	32	>256	2	0.5	0.06	>256	0.5	0.5
	G	5	PU86	16	32	128	64	>256	16	>256	4	0.25	0.125	256	≤0.125	1
	C	1	PUA7	16	32	>256	>256	>256	16	>256	4	0.5	0.06	128	0.5	0.5
	L	1	PU78	16	32	32	4	4	4	1	1	0.125	0.125	4	≤0.125	1
	R	1	PU4A22	64	128	>256	>256	>256	128	>256	128	16	4	4	2	1
FWH	P1	3	FW-F1	64	64	>256	>256	>256	64	>256	>256	16	4	8	2	0.5
BJ3	P1	26	BJ3-23	64	64	>256	>256	>256	64	>256	>256	16	4	8	8	1
	P3	6	BJ3-9	64	32	256	64	8	16	>256	>256	16	4	8	4	1
BJH	P2	8	BJ55	32	32	128	256	>256	64	>256	>256	>32	16	8	16	0.5
	P2	2	BJ52	32	32	128	32	16	32	4	2	32	8	8	8	1
DLH	A2	2	DL34	32	32	128	32	64	8	>256	>256	>32	8	4	8	0.5
NJH	A4	2	NJ59	16	16	>256	32	>256	128	>256	>256	>32	8	8	4	0.5
ZJH	A3	9	ZJ32	64	32	>256	128	64	32	>256	>256	>32	16	16	16	1
	T1	4	ZJ58	16	32	>256	32	16	32	>256	64	>32	>32	8	16	1
	T1	8	ZJ36	16	16	128	32	16	16	1	2	16	4	8	32	1
	T2	3	ZJ49	32	32	>256	64	32	32	>256	256	16	4	8	32	1
CYH	E	4	CY7	64	64	>256	>256	>256	32	>256	>256	>32	>32	4	0.5	0.5
	F	5	CY6	32	32	>256	>256	>256	16	>256	32	1	0.12	>256	0.25	0.5
GZH	M	6	ZS15	64	64	>256	32	16	4	256	256	32	4	>256	0.25	1
	N	12	ZS5	64	128	>256	>256	>256	32	>256	>256	>32	>32	256	8	0.5
FZH	Q	1	FZ4A18	16	16	64	>256	>256	32	128	32	0.5	0.12	4	0.5	1
	V	1	FZ84	16	16	64	>256	>256	32	>256	>256	>32	>32	128	4	0.5
JNH	U	2	JN45	32	16	256	128	>256	32	>256	128	2	0.5	>256	0.5	1

[a] CYH; Beijing Chaoyang Hospital; GZH, Guangzhou Zhongshan No. 1 Hospital; FZH, Fuzhou Xiehe Hospital; JNH, Shandong Qilu Hospital; DLH, Dalian No. 1 Medical College Hospital; NJH, Nanjing Provincial People Hospital.

[b] IPM, imipenem; MEM, meropenem; PTZ, piperacillin-tazobactam; FEP, cefepime; CAZ, ceftazidime; CSL, cefoperazone-sulbactam (2:1); GEN, gentamicin; AMK, amikacin; CIP, ciprofloxacin; LVX, levofloxacin; RIF, rifampin; MIN, minocycline; POL, colistin.

[c] The CLSI breakpoint for cefoperazone was used for cefoperazone-sulbactam in this study.

Sequencing of the entire $bla_{\text{OXA-51}}$-like gene of the 18 representative *A. baumannii* isolates revealed the presence of $bla_{\text{OXA-66}}$ (Table 4). The remaining 34 isolates, which did not carry $bla_{\text{OXA-51}}$-like genes, were not identified as *A. baumannii* by ARDRA.

One isolate of clone L (PU78, identified as *Acinetobacter* genospecies 3) was positive for a $bla_{\text{OXA-24}}$-like gene. Sequencing of the entire $bla_{\text{OXA-24}}$-like gene of PU78 indicated the presence of $bla_{\text{OXA-72}}$.

Seven isolates of clone T (identified as *A. baumannii*) and 1 isolate of clone Q (FZ4A18, identified as *A. phenon* 6/ct 13TU) carried the $bla_{\text{OXA-58}}$ gene. Similarly, one group of PFGE T1-type isolates, represented by ZJ36, carried the OXA-58 gene; however, the other isolates of the same PFGE subtype, represented by ZJ58, did not carry this gene (Table 4).

Isolate FZ4A18 was positive for bla_{IMP} and gave a 1.3-kb PCR amplicon for class 1 integrons that contained $bla_{\text{IMP-8}}$ and *aacA6*. The bla_{VIM}, $bla_{\text{CTX-M}}$, bla_{GES}, and bla_{VEB} genes were not detected in any of the 221 isolates, while 77.8% of the isolates carried a bla_{PER} gene, 69.2% a bla_{TEM} gene, and 5.4% a bla_{SHV} gene. Among 26 representative isolates, 14 carried $bla_{\text{PER-1}}$, 11 $bla_{\text{TEM-1}}$, and 1 a bla_{SHV}-like gene (Table 4). The isolates with the bla_{PER} gene were more resistant to ceftazidime and cefepime than the non-PER-1 producers.

IS*Aba1* was sought in all of the 26 representative isolates in Table 4. Only 2 isolates (PU78 and FZ4A18) were negative. All 22 isolates with $bla_{\text{OXA-23}}$ gave a PCR amplicon of ca. 1.6 kb with use of the IS*Aba1* forward primer and the $bla_{\text{OXA-23}}$ reverse primer. Among the 3 isolates with $bla_{\text{OXA-58}}$ (ZJ58, ZJ49, and FZ4A18), none gave a band in the PCR with use of the IS*Aba1*F and the $bla_{\text{OXA-58}}$ reverse primers. PCRs carried out using the IS*Aba1* forward primer and $bla_{\text{OXA-51}}$-like reverse primers, performed on the isolates with $bla_{\text{OXA-66}}$, failed to give a band of ca. 1.2 kb.

Integron detection. Class 1 integrons were sought for in the 26 representative isolates. Table 4 shows the 11 different structures of class 1 integrons that were found in these isolates. Overall, integrons were less common in *A. baumannii* than in the other *Acinetobacter* spp. Clone A subtypes of *A. baumannii* harbored integrons of different structures, while most of the clone P isolates did not carry class 1 integrons, except for the isolates from one hospital (BJ3). The most common gene cassettes in the integrons contained resistance determinants to aminoglycosides (*aacA4*, *aadA1*, and *aacA6*), to rifampin (*arr3*), and to chloramphenicol (*catB8*). The gene *arr3* was more common in non-*A. baumannii* representatives (Table 4).

Plasmid profiles and Southern blot analysis. Plasmid DNA was extracted from the 26 representative isolates and from the other OXA-58-producing *Acinetobacter* sp. isolates. The isolates with identical plasmid profiles, including isolates PU4A1, PU73, FW-F1, and BJ3-9 (Table 4), belonged to the same PFGE patterns. However, isolates of the same clone did not always contain the same plasmid profiles.

Seven isolates of *A. baumannii* clone T, possessing the $bla_{\text{OXA-58}}$ gene, had similar plasmid profiles. Southern hybridization analysis showed that the $bla_{\text{OXA-58}}$ gene was located on

TABLE 4. Genetic characterization of 26 representative clonal isolates at different hospitals

Isolate	PFGE clone	Genomic identification	Multiplex PCR result[a] for:				PCR result[a] for:				β-Lactamase types	Respective pIs of β-lactamases	Occurrence of IS*Aba1*[a]	Gene cassette in class 1 integrons	Plasmid profile(s) (kb)
			OXA-24 like	OXA-51 like	OXA-23 like	OXA-58 like	PER	IMP	TEM	SHV					
PU4A1	P1	*A. baumannii*	−	+	+	−	+	−	+	−	AmpC, OXA-66, OXA-23, PER-1, TEM-1	>9.0, 7.0, 6.7, 5.6, 5.4	+		10.0, 5.4
PU73	P1	*A. baumannii*	−	+	+	−	+	−	+	−	AmpC, OXA-66, OXA-23, PER-1, TEM-1	>9.0, 7.0, 6.7, 5.6, 5.4	+		10.0, 5.4
FW-F1	P1	*A. baumannii*	−	+	+	−	+	−	+	−	AmpC, OXA-66, OXA-23, PER-1, TEM-1	>9.0, 7.0, 6.7, 5.6, 5.4	+		10.0, 5.4
BJ3-23	P1	*A. baumannii*	−	+	+	−	+	−	+	−	AmpC, OXA-66, OXA-23, PER-1, TEM-1	>9.0, 7.0, 6.7, 5.6, 5.4	+	*aacC1-aadA1*	
BJ55	P2	*A. baumannii*	−	+	+	−	+	−	+	−	AmpC, OXA-66, OXA-23, PER-1, TEM-1	>9.0, 7.0, 6.7, 5.6, 5.4	+		
BJ52	P2	*A. baumannii*	−	+	+	−	−	−	+	−	AmpC, OXA-66, OXA-23, TEM-1	>9.0, 7.0, 6.7, 5.4	+		
BJ3-9	P3	*A. baumannii*	−	+	+	−	−	−	−	−	AmpC, OXA-66, OXA-23, TEM-1	>9.0, 7.0, 6.7, 5.4	+	*aacC1-orfx1-orfx2-orfx3*	10.0, 5.4
PUA1	A1	*A. baumannii*	−	+	+	−	+	−	+	−	AmpC, OXA-66, OXA-23, PER-1, TEM-1	>9.0, 7.0, 6.7, 5.6, 5.4	+	*arr3-orfx*	
DL34	A2	*A. baumannii*	−	+	+	−	−	−	−	−	AmpC, OXA-66, OXA-23, TEM-1	>9.0, 7.0, 6.7, 5.4	+	*orfx-aadA1*	
ZJ32	A3	*A. baumannii*	−	+	+	−	−	−	−	−	AmpC, OXA-66, OXA-23, TEM-1	>9.0, 7.0, 6.7, 5.4	+	*aacA4-catB8-aadA1*	5.9
NJ59	A4	*A. baumannii*	−	+	−	−	−	−	+	−	AmpC, OXA-66, TEM-1	>9.0, 7.0, 5.4	+	*aacA4-catB8-aadA1*	50.0
ZJ58	T1	*A. baumannii*	−	+	+	+	−	−	−	−	AmpC, OXA-58, OXA-66, OXA-23, TEM-1	>9.0, 7.2, 7.0, 6.7, 5.4	+		51.0, 12.4, 5.9
ZJ36	T1	*A. baumannii*	−	+	+	−	−	−	−	−	AmpC, OXA-66, OXA-23, TEM-1	>9.0, 7.0, 6.7, 5.4	+		84.0, 19.0, 8.5
ZJ49	T2	*A. baumannii*	−	+	+	+	−	−	−	−	AmpC, OXA-58, OXA-66, OXA-23, TEM-1	>9.0, 7.2, 7.0, 6.7, 5.4	+		51.0, 12.4, 5.9
PUA6	B	*A. phenon* 5	−	−	+	−	+	−	−	−	AmpC, OXA-23, unknown, PER-1, TEM-1	>9.0, 6.7, 6.0, 5.6, 5.4	+	*arr3-aacA4*	
PU86	G	*A. phenon* 6/ct 13TU	−	−	+	−	−	−	−	−	AmpC, OXA-23, TEM-1	>9.0, 6.7, 5.4	+	*arr3-orfx*	12.0, 6.7, 5.4, 4.3, 2.6
PUA7	C	Genospecies 3	−	−	+	−	+	−	−	−	AmpC, OXA-23, PER-1, TEM-1	>9.0, 6.7, 5.6, 5.4	+	*arr3-aacA4*	135.0, 62.0, 28.0, 12.0, 7.6, 5.2
PU78	L	Genospecies 3	+	−	−	−	−	−	−	−	OXA-72, TEM-1	8.0, 5.4	−		
PU4A22	R	*A. baumannii*	−	+	+	−	+	−	+	−	AmpC, OXA-66, OXA-23, PER-1, TEM-1	>9.0, 7.0, 6.7, 5.6, 5.4	+		
CY7	E	*A. baumannii*	−	+	+	−	+	−	+	−	AmpC, OXA-66, OXA-23, PER-1, TEM-1	>9.0, 7.0, 6.7, 5.6, 5.4	+	*arr3-orfx*	
CY6	F	Genospecies 13TU	−	−	+	−	+	−	−	−	AmpC, OXA-23, unknown, PER-1, TEM-1	>9.0, 6.7, 6.0, 5.6, 5.4	+	*arr3-aacA6*	152.0, 77.0, 64.0, 2.8
ZS15	M	Genospecies 10/11	−	−	+	−	−	−	−	−	AmpC, OXA-23, TEM-1	>9.0, 6.7, 5.4	+	*arr3-catB8*	7.6, 5.4, 2.0
ZS5	N	Genospecies 10/11	−	−	+	−	+	−	−	+	AmpC, SHV-like, OXA-23, PER-1, TEM-1	>9.0, 7.4, 6.7, 5.6, 5.4	+	*arr3-aacA4*	145.0, 52.0, 28.0, 13.0, 5.5, 4.8, 1.9
FZ4A18	Q	*A. phenon* 6/ct 13TU	−	−	−	+	+	+	−	−	AmpC, IMP-8, OXA-58, PER-1	>9.0, 8.2, 7.2, 5.6	−	$bla_{IMP\text{-}8}$-*aacA6*	70.5, 8.0
FZ84	V	*A. baumannii*	−	+	−	−	−	−	+	−	AmpC, OXA-66, TEM-1	>9.0, 7.0, 5.4	+		121.0, 34.0, 18.0, 7.5, 4.8
JN45	U	*A. baumannii*	−	+	+	−	+	−	−	−	AmpC, OXA-66, OXA-23, PER-1, TEM-1	>9.0, 7.0, 6.7, 5.6, 5.4	+		>182.0, 87.0, 24.0, 10.0, 5.6, 4.4

[a] +, present; −, absent.

a plasmid of approximately 51 kb in 7 isolates and, apparently, on the chromosome in isolate FZ4A18 (data not shown).

DISCUSSION

Recent reports of hospital outbreaks have documented the spread of imipenem-resistant *Acinetobacter* spp. (7, 9, 14). The results of this study show that clonal spread was the main reason for the increasing trend in imipenem resistance at the different hospitals. Patient transfer and hospital staff contact may have enhanced the spread of imipenem-resistant *Acinetobacter* sp. among different wards and different hospitals. Early recognition of the presence of imipenem-resistant *Acinetobacter* sp. clones is necessary in order to prevent their spread within the hospital environment.

Clonal outbreaks due to *A. baumannii* strains producing the OXA-23 carbapenemase have been reported in Brazil (9) and, more recently, in Korea (13) and France (16). This study found that OXA-23 was the most prevalent carbapenemase among imipenem-resistant isolates at multiple centers in China. We also found that the OXA-23 enzyme was produced by several different *Acinetobacter* species. Moreover, all of the representative OXA-23 producers carried the chromosomal $bla_{\text{OXA-23}}$ gene adjacent to the insertion element IS*AbaI*.

The OXA-51-like subgroup shares less than 63% amino acid identity with other class D enzymes (2, 11). The results of this study supported the proposal that $bla_{\text{OXA-51}}$-like genes are ubiquitous in *A. baumannii* but are not found in other *Acinetobacter* spp. (22).

OXA-58 was first found in France, in 2003, and in Turkey, with $bla_{\text{OXA-58}}$-bearing plasmids spreading among multiple clones of *Acinetobacter* spp. (23). A recent study reported that $bla_{\text{OXA-58}}$ was geographically widespread in three continents over a 10-year period (8). In contrast, the results of this study showed that $bla_{\text{OXA-58}}$ was prevalent only in Hongzhou City in eastern China. However, due to its plasmidic location, the distribution of this gene in isolates from other Chinese cities should be monitored.

PER-1 is an extended-spectrum β-lactamase active against penicillins, cefotaxime, ceftazidime, and aztreonam but with no significant activity against carbapenems (17). It has been widely detected in *Acinetobacter* spp. in Turkey and Korea (24, 29). In this study, about 78% of the Chinese imipenem-resistant *Acinetobacter* sp. isolates were found to produce a PER-1-like enzyme.

Integron typing and plasmid profiling are valuable tools for molecular epidemiology. In this study, the integron-borne gene cassette array *arr3-aacA4* was found in different species of *Acinetobacter*, suggesting horizontal gene transfer among species. Three isolates of clone P possessed identical plasmid patterns, as did 7 isolates of clone T, but most of the other isolates carried different integrons and contained different plasmid patterns, even within the same clonal cluster. Thus, isolates within a given genotype can acquire different accessory genetic elements, and unrelated clones may contain the same integrons or plasmids.

ACKNOWLEDGMENTS

We thank Wang Qingtao, Wang Feiyan, Jiang Wei, Hu Yunjian, Liao Kang, Huang Xinhong, Kong Qinglian, Wang Jing, Yu Yunsong, and Zhao Wangsheng for providing imipenem-resistant *Acinetobacter* spp. from their respective hospitals. We thank Wang Minggui and Xu Xiaogang, Institute of Antibiotics, Huashan Hospital, for kindly sending *E. coli* V517 containing plasmid R1 and *E. coli* J53 containing plasmid R27 and calculating the sizes of the plasmids. We are indebted to Timothy R. Walsh, Cardiff University, and David S. Perlin, Public Health Research Institute at NJMS-UMDNJ, for critically reading and editing the manuscript.

This work was supported by grant 30500023 from the National Natural Science Foundation of China.

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